DETAILED ACTION

1. Applicant's amendment filed April 14, 2011 is acknowledged. Claims 1 and 31 (currently amended), 2-13, 15-21 and 26-30 (original) and 42 and 43 (newly added) will be examined on the merits. Claims 14, 22-25 and 32-41 have been canceled.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1-10, 12, 13, 15-21, 26-31, 42 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yang et al. (Anal. Biochem. (1998) 259:272-274,

listed on IDS of 9/15/2006) in view of Livak et al. (U.S. Patent Pub. No. 2005/0053979) as evidenced by Cetojevic-Simin et al. (Archive of Oncology (2001) 9:33-37).

With regard to claims 1, 12, 13, 15-17, 19-21, 26-28, 31 and 43, Yang teaches a method for the analysis of a target sequence in a first sample (detection of human p53 mutations in samples was performed with an assay based on using labeled fluorescence resonance energy transfer (FRET) probes, p. 272, column 1, lines 8-12), said method comprising:

contacting the first sample with a mixture (samples included synthetic oligonucleotides containing DNA sequences corresponding to the wild-type and mutant forms of p53, p. 272, column 2, lines 30-33 and Figure 1, top and bottom sequence duplexes) comprising:

a mixture of probe A and probe B (fluorescently labeled pyrimidine-rich probes were prepared by automated DNA synthesis, p. 272, column 2, lines 33-37), wherein:

i) Probe A is directed to a region of the target sequence and is labeled with a fluorophore at the end which, upon hybridization is closest to the adjacent target region for Probe B (probe-2 is labeled with a donor moiety, 5-carboxyfluorescein (FL) at the 5' end and binds to form a DNA triplex to both the wild-type and mutant sequences, p. 272, column 1, lines 23-29 and column 2, 43-47 and Figure 1, middle right probe binding to right region of target);

ii) Probe B co-hybridizes to a region of the target sequence adjacent to the target region of Probe A and is labeled with a quencher which, upon hybridization is closest to the adjacent target region for Probe A (probe-1 is labeled with an acceptor moiety,

tetramethylrhodamine (TMR), at the 3' end and binds to form a DNA triplex to the wild-type sequence but only pairs with 7 of 12 bases in the mutant sequence, p. 272, column 2, lines 23-26 and 43-47 and Figure 1, middle left probe binding to left region of target), and

measuring fluorescence following co-hybridization of Probe A and Probe B to the target sequence, under suitable hybridization conditions, wherein the presence or amount of target sequence present in the first sample can be negatively correlated with the fluorescence of the fluorophore on Probe A (the presence of the wild-type sequence causes a decrease in fluorescence of the fluorescein-labeled probe 2, indicated by a decrease in emission at 520 nm, resulting from efficient binding of the quencher probe-1, p. 273, column 1, lines 17-24 and Figure 2, top panel).

With regard to claim 2, Yang teaches a method wherein the fluorescence is measurably different than a control (no decrease in emission at 520-nm is noted with the mutant target sequence, p. 273, column 1, lines 24-25 and Figure 2, top panel).

With regard to claim 3, Yang teaches a method wherein the control is a positive control, a negative control, a no target control or a second sample (the mutant sequence serves as a negative control since no energy transfer is observed compared with the wild-type sequence, p. 273, column 1, lines 17-25 and Figure 2, top panel).

With regard to claim 4, Yang teaches a method wherein the fluorescence is taken to be indicative of an increase in target concentration, a structural difference between sample and control target sequences or a change in the state of the target sequence (the decrease in fluorescence noted in the presence of the wild-type sequence is

indicative of presence of a sequence that is deleted in the mutant sequence, and therefore the quencher probe binds efficiently to the wild-type but not the mutant target, p. 272, column 2, lines 23-29 and p. 273, column 1, lines 17-25).

With regard to claims 5 and 7, Yang teaches a method wherein the structural difference between sample and control target sequences is at least one of a nucleobase insertion, nucleobase deletion, genetic polymorphism, splice variation, amplification or mutation (the structural difference between the wild-type and mutant target sequences is an 8-bp deletion at the third position of codon 285 within exon 8 of the human p53 gene, p. 272, column 2, lines 7-15).

With regard to claim 6, Yang teaches a method wherein the change in target state is due to at least one of degradation, methylation, folding, hybridization, or association of the target sequence with protein (the p53 deletion results in differential hybridization of the quencher probe-1 with the mutant sequence versus the wild-type sequence, p. 273, column 1, lines 17-25 and also see Figures 1 and 2).

With regard to claim 8, Yang teaches a method wherein the method is used to detect target sequence in a closed tube (homogeneous) assay (the FRET-based probe binding assay used to distinguish wild-type from mutant target sequences is performed in a spectrophotometer such that after the dye-labeled probes are allowed to equilibrate with the unlabeled target DNA solutions, the emission intensities are measured in a closed tube format, p. 273, column 1, lines 4-14).

With regard to claim 9, Yang teaches a method wherein the method is used to detect a nucleic acid comprising a target sequence wherein said nucleic acid has been

synthesized or amplified in a reaction occurring in the closed tube (homogeneous) assay (target DNA may be synthesized to construct duplex target models corresponding to the wild-type and 8-bp deletion mutant forms of p53, p. 272, column 2, lines 30-33 and Figure 1, top and bottom sequence duplexes).

With regard to claim 10, Yang teaches a method wherein preferred nucleic acid synthesis or nucleic acid amplification reactions are selected from the group consisting of: Polymerase Chain Reaction (PCR), Ligase Chain Reaction (LCR), Strand Displacement Amplification (SDA), Transcription-Mediated Amplification (TMA), Rolling Circle Amplification (RCA) and Q beta replicase (target samples may also be prepared by PCR amplification and hybridized to the dye-labeled probes to detect mutant or wild-type sequences, p. 274, lines 13-35).

With regard to claim 18, Yang teaches a method wherein the method is used to diagnose a condition of medical interest (the FRET-based assay was applied to screen clinical DNA samples, p. 274, column 1, lines 3-4).

With regard to claims 29 and 30, Yang teaches a method wherein the sample comprises a target sequence which is indicative for a genetically based disease or is indicative for a predisposition to a genetically based disease or associated with a disease selected from the group consisting of 5-Thalassemia, sickle cell anemia, Factor-V Leiden, cystic fibrosis and cancer related targets such as p53, pIO, BRC-1 and BRC-2 (the FRET-based assay was applied to screen clinical DNA samples obtained from patients diagnosed pathologically with hepatocellular carcinoma using primers and probes targeted to exon 8 of the p53 gene, p. 274, column 1, lines 3-35).

However, Yang does not disclose methods wherein the target or probes are immobilized to a surface such as an array or wherein one or both probes are blocking probes or linear beacons and/or comprise PNA or LNA. Yang also does not disclose detecting, identifying or qualifying the presence or amount of one or more species or types of a microorganism in the sample, wherein the method is used to determine the effect of antimicrobial agents on the growth of one or more microorganisms in the sample. Yang also does not disclose a method wherein the target sequence in a forensic technique such as prenatal screening, paternity testing, identity confirmation or crime investigation, or wherein the target sequence is rRNA.

With regard to claims 1 and 43, Livak teaches methods for design and making of oligonucleotide probes comprising universal base analogues for use in nucleic acid hybridization assays, and may also comprise other oligomeric structures such as locked nucleic acids (LNA) and peptide nucleic acids (PNA) (see Abstract and paragraph 36, lines 1-20 and paragraph 88, lines 1-11). The probes may be labeled with energy transfer labels to form FRET probes suitable for use in real-time PCR analysis or other probe-hybridization based assays (paragraph 187, lines 1-6 and paragraph 222, lines 1-11). Energy-transfer probes can be provided as pairs of probes with donor and acceptor fluorophore moieties attached to ends of the same probe or on separate probes, wherein changes in a detectable signal from the donor or acceptor moieties result from hybridization, usually as an increase in measurable fluorescence (paragraph 216, lines 1-11, paragraph 223, lines 1-12, paragraph 224, lines 1-12 and paragraph 225, lines 1-23).

With regard to claims 12, 13, 26 and 27, Livak teaches a method wherein the method further comprises contacting the sample with at least one blocking probe and/or hybridizing the blocking probe to non-target sequences, wherein the blocking probe is PNA or LNA (probes of the invention may also be used as unlabeled blocking probes to bind non-target sequences more favorably than labeled probes, paragraph 281, lines 1-4 and 8-11 and paragraph 281, lines 1-9; probes of the invention may comprise PNA or LNA, though blocking probes are preferentially PNA probes, paragraph 36, lines 15-20 and paragraph 281, lines 4-6).

With regard to claims 15-17, Livak teaches a method wherein the method further comprises detecting, identifying or qualifying the presence or amount of one or more species or types or a taxonomic group of a microorganism in the sample, and wherein the method is used to determine the effect of antimicrobial agents on the growth of one or more microorganisms in the sample (methods of the invention are used for identification of pathogens such as viruses, bacteria and eucarya in clinical samples, and for measuring the effects of products used to treat infected subjects during therapy monitoring and in pharmacogenomic and pharmacogenetic studies, paragraph 276, lines 1-17 and paragraph 277, lines 1-8).

With regard to claims 19-21, Livak teaches a method wherein the target sequence or Probe A is immobilized to a surface, such as an array (probes may be immobilized to a solid support to form an array or chip of oligomers, paragraph 264, lines 1-9 and paragraph 265, lines 1-15, and one or more target sequences are captured on the array by hybridization to complementary probes at specific locations for

identification of the target by determining the location of a detectable signal generated on the array, paragraph 269, lines 1-13).

With regard to claim 28, Livak teaches a method wherein at least one of the probes is a linear PNA beacon (probes may comprise a PNA backbone structure, paragraph 157, lines 1-14, and may also be labeled with suitable energy transfer pairs to form a linear or molecular beacon to detect target nucleic acids by hybridization, paragraph 221, lines 1-9).

With regard to claim 31, Livak teaches a method wherein the target sequence in a forensic technique such as prenatal screening, paternity testing, identity confirmation or crime investigation (the methods can be used for identification of nucleic acid sequences in biological organisms, such as is useful in paternity testing and human identification, paragraph 7, lines 1-6 and paragraph 276, lines 1-3).

With regard to claim 42, Livak teaches a method wherein the target sequence is rRNA (target sequences may be composed of DNA or RNA, including forms of RNA, such as rRNA, that represent converted genetic information encoded in the genomic nucleotide sequence of a chromosome, paragraph 63, lines 5-9 and paragraph 73, lines 1-6).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Yang and Livak since both references teach methods of nucleic acid hybridization using labeled probes, and measuring hybridization either by negative (Yang) or positive (Livak) changes in fluorescence of the donor moiety of a pair of FRET probes. Thus, an ordinary

practitioner would have been motivated to combine the methods of using FRET-labeled probes of Livak that may also comprise LNA or PNA in the FRET-based methods of Yang since probes containing these non-natural modifications possess exceptional hybridization specificity and affinity, and are not known to be a substrates for enzymes such as polymerases or nucleases (Livak, paragraph 158, lines 4-7 and paragraph 159, lines 1-4). The probes of Livak are also useful as unlabeled blocking probes since such probes are able to suppress the binding of labeled probes to non-target sequences that might be present in an assay and otherwise interfere with the performance of the assay (Livak, paragraph 282, lines 14-20). Furthermore, the probes of Livak are useful for detecting a variety of nucleic acid targets, including those of microorganisms or in samples used in paternity testing and human identification (paragraph 7, lines 1-6 and paragraph 276, lines 1-17). Any of these probes may be designed with energy transfer labels attached to the probe termini as taught by Yang for use in nucleic acid hybridization assays that measure successful hybridization by the decrease in fluorescence of the donor moiety of one of the FRET probes. Finally, as evidenced by Cetojevic-Simin, PNA probes are useful for detecting target sequences either through formation of triplexes wherein DNA duplexes may form strand displacement complexes or triplex formations upon interaction with a double-stranded DNA target as well as by forming complexes with single-stranded nucleic acid targets by Watson-Crick base pairing (see Cetojevic-Simin, p. 34, column 1, lines 8-30 and Figure 2), and therefore would be compatible with the triplex-based methods of Yang or the duplex-based methods of Livak.

5. Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yang et al. (Anal. Biochem. (1998) 259:272-274) in view of Livak et al. (U.S. Patent Pub. No. 2005/0053979) 9:33-37) as evidenced by Cetojevic-Simin et al. (Archive of Oncology (2001), as applied to claims 1-10, 12, 13, 15-21, 26-31, 42 and 43 above, and further in view of Johansen et al. (U.S. Patent No. 6,441,152).

Yang and Livak, as evidenced by Cetojevic-Simin, teach the limitations of claims 1-10, 12, 13, 15-21, 26-31, 42 and 43, as discussed above. However, none of the references disclose methods wherein the PCR reaction is an asymmetric PCR reaction.

Johansen teaches methods for detecting nucleic acid targets after binding to matrices containing non-nucleotide probes such as PNA probes (see Abstract and column 8, lines 60-65). Johansen further teaches that the binding of nucleic acids to the matrices may be monitored by closed-tube PCR assays such as self-indicating assays that measure fluorescence within the closed reaction tube after amplification and binding to complementary bound probes (column 35, lines 1-14). Johansen further teaches that the amplification may be asymmetric PCR to favor production of a significant excess of single-stranded nucleic acid (column 35, lines 53-60).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Yang, Livak and Johansen since Johansen teaches a method for amplification of target nucleic acids that is highly compatible with the FRET probes taught by Yang and Livak for detection of mutant and wild-type target sequences. Thus, an ordinary practitioner would have been motivated

to use the methods for asymmetric PCR in preparation of target nucleic acids which can then be detected in a closed tube assay using the FRET probes of Yang or Livak, including PNA probes. The asymmetric PCR method taught by Johansen favors production of a significant excess of single-stranded nucleic acid such that "it is possible to choose which strand of the amplicon are preferentially amplified by judicious adjustment of the ratio of 5' and 3' primers" (Johansen, column 35, lines 53-60). Thus, probes may be designed with energy transfer labels attached to the probe termini as taught by Yang and Livak that hybridize specifically to the preferentially amplified strand, which is then detected as a decrease in fluorescence of the donor moiety of one of the FRET probes.

Response to Arguments

6. Applicant's arguments filed April 14, 2011 have been fully considered but they are not persuasive.

Applicant argues that the objection of claims 1-31 for minor informalities has been overcome based on amendments to the claims. The Examiner agrees and therefore the objection is withdrawn.

Applicant argues that the rejection of claim 31 under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention should be withdrawn based on

an amendment to the claim to include an active step in the claim. The Examiner agrees that the amendment clarifies the rejected claim and the rejection is thus withdrawn.

Applicant then argues that the rejection of claims 1-10, 18, 29 and 30 under 35 U.S.C. § 102(b) as being anticipated by Yang et al. (Anal. Biochem. (1998) 259:272-274) should be withdrawn since the reference no longer teaches all the limitations of the claims as amended. In particular, Applicant argues that Yang does not disclose methods for the analysis of a target sequence wherein Probe A and Probe B are PNA probes. Applicant further argues that Yang teaches a method based on triplex formation that involves Hoogsteen base-pairing rather than the use of probes that bind by Watson-Crick base pairs as required by the present invention. The Examiner agrees that Yang does not teach a method for the analysis of a target sequence wherein Probe A and Probe B are PNA probes. Therefore, the 102(b) rejection is withdrawn.

However, the Examiner asserts that neither the claims nor the disclosure require that PNA probes function only through interaction with single-stranded target strands, such as by Watson-Crick base pairing. Therefore, a new 103(a) rejection of claims 1-10, 12, 13, 15-21, 26-31, 42 and 43 as being obvious over Yang in view of Livak et al. (U.S. Patent Pub. No. 2005/0053979) as evidenced by Cetojevic-Simin et al. (Archive of Oncology (2001) 9:33-37) is made since Livak teaches that pairs of PNA probes may be used for analysis of target sequences by FRET, while Cetojevic-Simin provides evidence that PNA probes are useful for binding to target sequences both by formation of triplex complexes, as well as by formation of duplex complexes (see above). One of skill in the art would have been aware at the time of the invention that PNA probes may

be used as FRET probe pairs for detection of single-stranded targets, as taught by Livak and evidenced by Cetojevic-Simin, but could also be used to form complexes with double-stranded DNA targets through triplex formation, as evidenced by Cetojevic-Simin. Thus, the general practitioner could choose to design FRET probe pairs for either type of target binding, and detect the binding by negative correlation of the fluorescence as taught by Yang.

Applicant then argues that the rejection of claim 11 over Yang in view of Johansen et al. (U.S. Patent No. 6,441,152) should be withdrawn since the references no longer teach all the limitations of the claims as amended. Applicant's additional arguments with respect to the previous rejections of record regarding Yang and Johansen have been noted, but are moot in view of the rejection of the claims based on new grounds necessitated by the amendments (see above).

Applicant then argues that the rejection of claim 12-17, 19-28 and 31 over Yang in view of Livak et al. (U.S. Patent Pub. No. 2005/0053979) should be withdrawn since the references no longer teach all the limitations of the claims as amended. As discussed above, claims 1-10, 12, 13, 15-21, 26-31, 42 and 43 are now rejected under 35 U.S.C. § 103(a) as being obvious over Yang in view of Livak et al. (U.S. Patent Pub. No. 2005/0053979) as evidenced by Cetojevic-Simin. Applicant further argues that Livak, while teaching the use of pairs of FRET probes that may also be PNA probes, does not teach the use of the probes to provide a negative correlation between signal and the amount of target. The Examiner asserts that one of skill in the art will recognize that pairs of FRET-labeled probes that bind at adjacent sites (kissing probes) can be

used in assays based on negative or positive correlation of fluorescence (for example, see Yang, see Figure 2). Therefore, the teachings of Yang and Livak, and the evidenced provided by Cetojevic-Simin, are now applied to the new rejection.

Summary

7. Claims 1-13, 15-21, 26-31, 42 and 43 are rejected. No claims are allowable.

Conclusion

8. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Correspondence

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David C. Thomas whose telephone number is 571-272-

3320 and whose fax number is 571-273-3320. The examiner can normally be reached on 5 days, 9-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/David C Thomas/
Examiner, Art Unit 1637

/Kenneth R Horlick/

Primary Examiner, Art Unit 1637